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Bacterial Type III Effectors Inhibit Cell Lifting by Targeting Integrin-Linked Kinase

Nicole Tegtmeyer[1] and Steffen Backert[1,*]
[1]University College Dublin, School of Biomolecular and Biomedical Sciences, Ardmore House, Belfield Campus, Dublin-4, Ireland
*Correspondence: steffen.backert@ucd.ie
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Many pathogenic bacteria are able to surmount exfoliation and colonize the host epithelium efficiently. In a recent article in *Nature*, Kim et al. (2009) show that *Shigella flexneri* OspE, a conserved type III effector, reinforces host cell adherence and prevents detachment of the infected cells by interacting with integrin-linked kinase.

The intestinal epithelium serves as an intrinsic barrier against microbial pathogens. The commensal microflora, innate immune responses, rapid cell turnover, and exfoliation of mucosal epithelial cells provide effective defense mechanisms against infections (Sansonetti, 2004; Radtke and Clevers, 2005; Ogawa et al., 2008). However, many pathogenic bacteria are able to surmount exfoliation and colonize the gastric epithelium efficiently. Some of these pathogens use sophisticated type III secretion systems (T3SSs) to inject virulence proteins into host cells, which then manipulate signaling cascades. Kim et al. (2009) show that *Shigella* use a previously unknown tactic to strengthen adhesion of infected cells to the underlying extracellular matrix (ECM). This is achieved by the T3SS effector protein OspE, which binds to integrin-linked kinase (ILK). The ILK-OspE complex stabilizes integrin-containing adhesion sites, reduces adhesion turnover, and suppresses the detachment of infected cells from the basolateral membrane, which ultimately enables the pathogen to maintain the infectious foothold. Since OspE is highly conserved among *Shigella*, enteropathogenic *Escherichia coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), *Citrobacter rodentium*, and *Salmonella* strains, these findings uncover a strategy that could be exploited to interfere with infections of many mucosal pathogens.

Shigella passes the epithelial cell barrier by transcytosis through M cells and encounters resident macrophages (Figure 1A). The bacteria evade killing within macrophages by inducing macrophage apoptosis. Released bacteria then invade neighboring epithelial cells from the basolateral side, move into the cytoplasm by inducing actin polymerization, and spread to adjacent cells (Figures 1A and 1B). This so-called invasive phenotype is linked to local but massive changes in the cell cytoskeleton at the site of bacterial internalization. The signaling cascades involved in this scenario are highly complex and require multiple bacterial effectors, such as the Ipa, Ipg, IcsA/VirG, and other proteins (Figure 1C). Kim et al. (2009) show that OspE secreted from *S. flexneri* reinforces host cell adherence to the basolateral membrane by interacting with ILK. ILK normally interacts with the cytoplasmic domains of β1- and β3-integrin receptors to stimulate numerous downstream signaling factors, such as AKT (also called protein kinase B [PKB]), GSK3β (glycogen synthase kinase-3 beta), or formation of the ILK-PINCH (LIMS1)-parvin (IPP) complex (Legate et al., 2006; McDonald et al., 2008).

The OspE-ILK interaction was confirmed in several assays (Kim et al., 2009), and OspE localized to focal adhesions as previously shown (Miura et al., 2006). To investigate the functional link between OspE and ILK in focal adhesion formation, ILK-deficient mouse embryonic fibroblasts (MEFs) and MEFs re-expressing different ILK constructs (wild-type, kinase-inactive, or kinase-active) were co-transfected with green fluorescent protein (GFP)-tagged OspE. In the absence of ILK, OspE was dispersed throughout the cytoplasm, whereas OspE colocalized with wild-type ILK at focal adhesions, indicating that the localization of OspE at focal adhesions requires ILK. Importantly, wild-type or kinase-active ILK cells transfected with OspE contained significantly more focal adhesions than cells expressing kinase-inactive ILK or ILK-deficient MEFs. Because OspE bound to the C-terminal region of wild-type and ILK mutants to similar degrees, the authors tested whether OspE affects ILK signaling. Interestingly, OspE did not change the activity of ILK downstream targets AKT and GSK3β nor the formation of the IPP complex. OspE also did not affect ILK stability. These results provided comprehensive evidence that OspE does not change ILK kinase activity. However, OspE boosted ILK to the membrane, indicating that OspE promotes membrane retention of ILK, whereas the ILK-OspE complex stabilizes and strengthens focal adhesion sites.

In healthy mammalian tissues, ILK exhibits a critical role in coupling the ECM to the actin cytoskeleton as well as signaling complexes, thereby modulating focal adhesion assembly and disassembly (Legate et al., 2006; McDonald et al., 2008). Therefore, it was proposed that OspE may affect early ECM adhesion and focal adhesion assembly. The results indicated that the OspE-ILK interaction interferes with focal adhesion disassembly. In the presence of OspE, phosphorylation of focal adhesion kinase (FAK) was markedly reduced in both wild-type and kinase-active ILK cells (Kim et al., 2009). The same was seen for tyrosine phosphorylation levels of paxillin and total cellular proteins. These results strongly indicated that the OspE-ILK complex interferes with focal adhesion disassembly and thereby dampens focal adhesion turnover in an ILK kinase domain-dependent manner. In addition, the surface levels of β1-integrin were 1.8-fold higher in both OspE-transfected wild-type and kinase-active ILK MEFs than in cells without OspE, and OspE

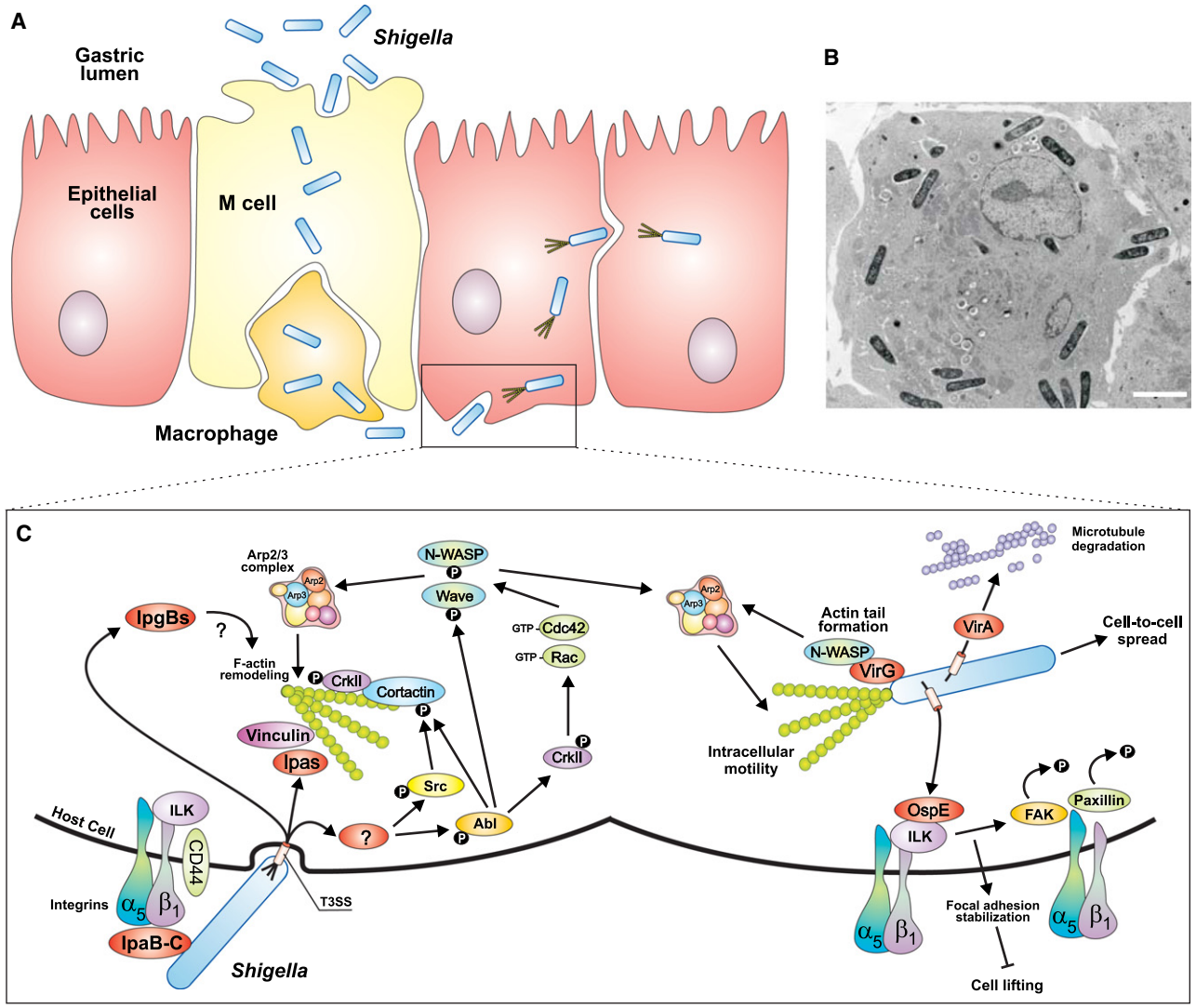

Figure 1. Infection Route and Molecular Signaling Involved in Invasion and Cellular Spread of *Shigella* spp

(A) *Shigella flexneri* passes the intestinal epithelial barrier by transcytosis through M cells and encounters resident macrophages. The bacteria evade degradation in macrophages by inducing their cell death. Bacteria are released and can invade the epithelial cells from the basolateral side, move into the cytoplasm by triggering actin polymerization, and spread to adjacent cells.

(B) Electron microscopic picture of an epithelial cell infected with wild-type *S. flexneri* (kindly provided by Michinaga Ogawa; University of Tokyo, Japan). Bar, 5 μm.

(C) Molecular signaling pathways involved in bacterial cell invasion and intracellular/intercellular movement of *S. flexneri* (Sansonetti, 2004; Ogawa et al., 2008). Bacteria released from dead macrophages can enter the surrounding enterocytes using a functional T3SS. In vitro studies with cultured cells have demonstrated that β_1-integrins and CD44 receptor may play a role in the initial contact of *Shigella* with the epithelial cell. T3SS-dependent injection of bacterial effector molecules (red) such as the Ipa proteins (IpaA–D) initiates actin-cytoskeletal and membrane remodeling processes that engulf the bacteria by macropinocytic ruffles. Exploring the process of Ipa proteins altering the host cytoskeleton to induce the uptake process revealed that a complex of IpaB and IpaC binds the $\alpha_5\beta_1$-integrin receptor and the hyaluron receptor CD44 and induces actin rearrangements at the site of bacterial attachment. Earlier work demonstrated that IpaA binds to the focal adhesion protein vinculin and induces recruitment of filamentous (F)-actin and depolymerization of actin stress fibers, but it was also shown that IpaA increases the activity of the small GTPase RhoA and decreases integrin affinity for ECM ligands by interfering with talin recruitment to the integrin's cytoplasmic tail. *Shigella* entry through the Ipa proteins further implicates the recruitment and activation of multiple cellular factors such as the tyrosine kinases FAK, Src, and Abl; cortactin; CrkII; Rac; or Cdc42, which mediate massive actin polymerization in the vicinity of the original cup via activation of Wave and the Arp2/3 complex (Nhieu et al., 2005; Backert et al., 2008; Ogawa et al., 2008; Parsot, 2009). In addition, a new class of GTPase-mimicking T3SS effector proteins (IpgB1 and IpgB2), which is also involved in invasion, has been recently discovered in *Shigella*. As soon as the bacterium is surrounded by a membrane vacuole within these epithelial cells, it disrupts the vacuolar membrane and escapes into the host cell cytoplasm. *Shigella* movement is then triggered by the bacterial surface protein VirG/IcsA. VirG/IcsA exhibits a high affinity to a major regulator of the actin polymerization machinery, N-WASP (neuronal Wiskott-Aldrich syndrome protein), which recruits and activates the Arp2/3 complex. The formation of actin tails pushes *Shigella* through the host cell cytoplasm, a process that is enhanced by secreting the T3SS effector protein VirA, which destroys surrounding microtubules. New work discussed here shows that the T3SS effector protein OspE binds ILK, induces the tyrosine dephosphorylation of FAK and paxillin, stabilizes integrin-containing adhesion sites, reduces adhesion turnover, and suppresses the detachment of infected cells from the basolateral membrane (Kim et al., 2009). In this way, *Shigella* can effectively multiply in the epithelial cell cytoplasm and move both intra- and intercellularly (Sansonetti, 2004; Ogawa et al., 2008; Parsot, 2009).

expression blocked cell motility. Taken together, these results indicate that OspE-ILK association interferes with focal adhesion disassembly and dampens integrin turnover by means of its effect on the phosphorylation of FAK, paxillin, and possibly other ILK target proteins. To investigate the biological significance of the findings, the authors identified tryptophan residue 68 (W68) in *Shigella* OspE to be crucial for binding to ILK. Interestingly, W68 is highly conserved among OspE orthologs encoded by EPEC, EHEC, *C. rodentium*, *S.* Typhimurium, and *S. enteritidis*. These OspE proteins were also demonstrated to interact with ILK and localize to focal adhesions, implying that OspEs play important roles in bacterial infection.

To establish the role of OspE in *Shigella* infection, HeLa cells were infected with *S. flexneri* wild-type and *ospE* mutants (Kim et al., 2009). Importantly, cells infected with Δ*ospE* or Δ*ospE*/*ospE*(W68A) but not Δ*ospE*/*ospE* wild-type detached from the culture plates, and ILK was dispersed in the cytoplasm. The ability of OspE to prevent cell detachment during bacterial infection was further confirmed by measuring the rounding up of HeLa or ILK-depleted HeLa cells infected with *S. flexneri* wild-type or Δ*ospE*. The data supported the view that OspE promotes membrane retention of ILK, whereas the OspE-ILK complex contributes to inhibition of focal adhesion disassembly. Indeed, the effect of OspE on epithelial integrity after bacterial infection was substantial. The ability of Δ*ospE* and Δ*ospE*/*ospE*(W68A) mutants to spread intercellularly was greatly impaired as compared to wild-type *S. flexneri*. Furthermore, infection of guinea pigs with wild-type *S. flexneri* resulted in severe inflammation, internal hemorrhaging, and diarrhea, whereas these pathogenic features were not prominent after Δ*ospE* or Δ*ospE*/*ospE*(W68A) inoculation. Finally, colonization by Δ*ospE* or Δ*ospE*/*ospE*(W68A) mutant *S. flexneri* in guinea pigs was significantly reduced as compared to wild-type bacteria. These data support the view that OspE plays an important role during *S. flexneri* infection in vitro and in vivo. Interestingly, recent data indicated that OspE is controlled by the transcriptional regulator MxiE of *S. flexneri* (Parsot, 2009). The MxiE dependency implies that the *ospE* gene is only expressed under intracellular conditions. Thus, OspE acts when *Shigella* has already entered the epithelial cells, which is an elegant mechanism to suppress the detachment of infected cells from the basolateral membrane and to ensure successful cell-to-cell spread of the bacteria (Figure 1C). Taken together, these findings uncover a strategy that could be exploited by *Shigella* and other mucosal pathogens expressing OspE orthologs. Discovery of small inhibitor molecules that may block the interaction of OspE with ILK could provide effective therapy for several bacterial infectious diseases.

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